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LETTERS TO THE EDITOR

Characterization of an Orthorhombic Crystal Form of Iron-containing Superoxide Dismutase from *Escherichia coli* B

Crystals suitable for X-ray diffraction studies of iron-containing superoxide dismutase from *Escherichia coli* have been grown using ammonium sulfate as the precipitant. The space group is $P2_12_12_1$ with $a = 81{\cdot}8$ Å, $b = 75{\cdot}2$ Å, $c = 71{\cdot}3$ Å. A dimer of $M_r = 40{,}000$ occupies the asymmetric unit.

Superoxide dismutases are metalloproteins which catalyze the reaction $2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$. These proteins, which have been isolated from both aerobic and strictly anaerobic organisms, require a metal ion for their activity and are readily classified on this basis. Thus eukaryotic cells generally possess two types of superoxide dismutase, one containing zinc and copper, and one having manganese as the essential cofactor. Prokaryotic cells have been found to have both iron and manganese-containing proteins (Michelson *et al.*, 1977; Beem *et al.*, 1976; Fridovich, 1975).

X-ray crystallographic analysis has yielded a three-dimensional structure for the bovine erythrocyte (Zn/Cu) superoxide dismutase (Richardson *et al.*, 1975) and work is in progress on the manganese-containing proteins from *Escherichia coli* and yeast mitochondria (Beem *et al.*, 1976), *Bacillus stearothermophilus* (Bridgen *et al.*, 1976; Smit *et al.*, 1977) and the iron-containing protein from *Pseudomonas ovalis* (Yamakura *et al.*, 1976; Petsko, personal communication).

Iron-containing superoxide dismutase from *E. coli* was first isolated by Yost & Fridovich (1973). A modified procedure (Slykhouse & Fee, 1976) was used to provide the starting material for the work described here. Crystals were grown at 4°C in a hanging droplet containing 10 μl of protein solution (5 to 10 mg/ml in 5mM-phosphate buffer, pH 7·5) and 5 μl of saturated ammonium sulfate. The siliconized glass coverslip carrying the droplet is sealed with silicone grease over a well containing 0·2 ml of a solution that is 40% saturated ammonium sulfate and 0·5 M-potassium acid phosphate (pH 5·0). A cloudy precipitate develops within a few minutes. Crystals grow within the precipitate and may be observed after two days. In three weeks, five to ten crystals of dimensions suitable for X-ray diffraction measurements (0·3 mm × 0·3 mm × 0·8 mm, and larger) may be harvested from the droplet. These crystals are not visually dichroic. Precession photographs and diffractometer measurements yielded the space group $P2_12_12_1$ with unit cell dimensions of $a = 81{\cdot}8$ Å, $b = 75{\cdot}2$ Å and $c = 71{\cdot}3$ Å. No other crystal modification has been observed.

Crystal density was measured by the Ficoll gradient procedure described by Westbrook (1976). The observed value of 1·175 g per cm^3 indicates that a dimer of $M_r = 40{,}000$ occupies the asymmetric unit. In addition, a value for V_m (Matthews, 1968) of 2·83 Å^3/dalton for the dimer is typical of protein crystals. We are currently pursuing the three-dimensional structure of this crystal form.

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